179

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Human Implications of Biological Discovery

Recent advances in medical and biological science, such as the heart transplantation technique by Norman Shumway and DNA replication in vitro by Arthur Kornberg, my colleagues at the Stanford Medical School, are indeed fabulous scientific advances. Without question these events hold great promise for the future of mankind. In the not too distant future, we will be able to artificially create and sustain life. And serious discussions continue in regard to our potential ability to use biochemistry to alter genetic structure and thereby change the minds and bodies of men. These dramatic possibilities and others as yet unmentioned promise the steady lengthening and improving of human life. However, I believe they also raise profound questions for our society: What is life? What is death? Who shall live and who shall die? Whose genes shall be altered and for what purpose? How long and under what conditions shall we prolong life? How far shall we go in creating artificial life, and what will be its status once it has been created?

A NATIONAL COMMISSION FOR CONTROLLING BIOLOGICAL SCIENCES?

Recently, Senator Walter F. Mondale, a Democrat from Minnesota, placed a rather sharp emphasis on the recent heart transplant operations:

> Because I believe it is imperative to deal with these crucial considerations as rationally and as publicly as possible, I intend to introduce a resolution early in the coming session of the United States Senate to establish a national commission on the ethical and social implications on health science research and development. This committee would study the meaning of health science development for this nation and the world, explore its moral and ethical implications and formulate ethical guidelines for its application, and make recommendations to the President and to the Congress for actions to insure that our social policies reflect and influence our technological advances. The Commission and its staff should represent a broad cross-section of disciplines: scientists, health practitioners, administrators, economists, educators, theologians, sociologists, philosophers, and attorneys.

In addition, if there is anyone left, the general American public must be heard if meaningful recommendations are to be made.

I quote this, because it represents the appearance in the legislative consciousness of the impact of current biological advance and its potential relationship to the human condition, to a degree that is unprecedented in our cultural history. In fact, these kinds of remarks lead me to a near reversal on the fundamental stance that I would have taken previously. Six months ago I would be exhorting giving some concern to the way in which the quality of life is likely to be influenced by biology, and asking you to think about biology as one of the politically oriented, socially scientific disciplines. The newspapers have done a very good job of wresting that job from my hands, and I now propose to do almost exactly the opposite: namely, to attempt to quiet some of the unwarranted and unnecessarily extreme extrapolations that might be made even beyond Senator Mondale's statement.

Let me say at the outset, by the way, that I do not think his commission is a good idea if it is going to address itself to the ethical and moral guidelines for applications of medicine. Congress is an excellent organization to the extent that it is representative of our society for the promulgation of laws, but I don't see how in the world any creature of the legislature can lay down moral and ethical guidelines and prescriptions. I would wonder, for example, how it would deal with such matters as private choices in areas such as abortion or contraception or any of a number of things that some people still regard as controversial.

On the other hand, there are urgent matters of law to which Congress should be addressing itself that have to do with at least some of the questions mentioned here. If there is to be an intelligent approach to them, we must particularize. We must get past the stage of throwing up our hands in awe or horror against the vague possibilities of future developments and try to isolate those concerns that we can frame in realistic terms and that represent real challenges to our existing legal system, if we are to have a healthy society.

"CREATION" OF LIFE IN TEST TUBE

Now the event that has focused so much attention on these concerns—or these events—has been the transplantation of the heart, as practiced in Capetown and at Stanford; and perhaps at a much more fundamental level, the announcements that have appeared in the scientific literature and have been widely echoed in the press concerning the accomplishments of my colleague, Arthur Kornberg, in the Department of Biochemistry. This has been described as the creation of life in the test tube, and has evoked all kinds of images of the way in which science is now going to modify our genes in the not too distant future. We wonder who will decide whose genes will be altered, in what condition, and so on.

Statements have also been made to the effect that we were about to learn how to make supermen by the application of biochemical genetic principles. Senator Mondale was rather alarmed about that, and I think most of us might be if we were faced with the reality.

RECENT GENETICS RESEARCH

I think perhaps I should first review the actual significance of these epochal contributions from the laboratory, as they do come from the work of one of my close friends and one of my most admired colleagues. I regard the experimental replication of DNA to be one of the most astounding intellectual achievements of the century. I think there has been a certain distortion of emphasis in the newspaper accounts of this work. It is not incorrect to speak about it as the creation of life, but I think we would have to be very careful in our definition of terms if we were to justify that description.

Almost 15 years ago Kornberg began to study the question of the way in which the cell replicates its DNA. At that time it was already reasonably clear—although the final evidence was perhaps not quite in—that DNA was the genetic material. That is to say, the information that prescribes the way in which an organism should develop, and which is present in the nucleus, in the chromosomes of every cell—there being a copy of the original nucleus that was in the original egg cell from which each one of us was derived—was in the form of a very complex organic molecule, deoxyribonucleic acid, or DNA.

Now geneticists have been studying DNA without knowing it for quite a few years. They have been dealing with "genes" at a much higher level of abstraction since their first recognition about 100 years ago in the pioneering work of Mendel, work that was actively ignored by his contemporaries for about 35 years; it had too mathematical and numerological a flavor to be convincing to a generation of biologists who refused to believe that rules of number could have any part at all in the workings of life. The view that living organisms must have rules of their own, and that we cannot apply the simple laws of chemistry and physics or mathematics, is one that has been stubbornly held by a certain fraction of biologists but one that has been constantly retreating against the onslaught of scientific advance. The resistance to the adoption of the theory of evolution and the resistance to the adoption of Mendel's account of the behavior of the genetic material were last century's contributions to that particular struggle. Much more recently we found geneticists working on the rules of inheritance in numerical terms and quite unwilling to discuss the actual material basis of the genetic material, and at least some geneticists stubbornly resisting some of the evidence that showed them that there were actually substantial molecules that could be invoked to account for the behavior of the genetic system. And that stubbornness, that unwillingness to reduce living systems to a materialistic framework, more than anything else accounts for the delay in the development of a chemistry of life. There were traces of it even in well-intentioned and hard-thinking

biologists in the thirties and forties that was expressed not so much in a studious opposition to new thought and new experimentation trying to find the biochemical basis of genetics, but more in ignoring it. Kornberg's major contribution—his major intellectual insight—was to regard the problem as soluble: One could discover an enzyme to account for the replication of genetic material, and one could attack that problem by the existing techniques of enzymology—that if one developed a biochemical assay for some steps in the replication of DNA, one could just track this down in the cell, try to purify the enzyme, try to determine the conditions under which it operated, and so on, and that such a program would have some hope of success. Very few of his contemporaries had the confidence that such a program was possible. I think most geneticists would have regarded him as hopelessly ambitious in attempting to perform a task such as the replication of a gene in the test tube.

In fact, it is quite remarkable that this feat was accomplished well before what for a long time had been regarded as a much more inevitable result: the artificial reproduction of proteins by an enzymatic system. The DNA preliminaries were worked out 5 to 10 years before a real understanding of the mechanisms of protein synthesis. However, about 11 or 12 years ago Kornberg reached the point in his investigations where he was able to publish a report on the isolation of an enzyme that he called DNA-polymerase, which had the essential properties of accepting a primer specimen of DNA that could be isolated from any particular kinds of cells, and that in the presence of the appropriate starting materials, new molecules of a DNA-like material would appear resembling that of the primer that was put in. This is quite unlike most enzymatic systems whose whole information with regard to what kind of product they make is inherent in the enzyme. If I begin with starch and one enzyme, I know I am going to obtain glucose-phosphate; if I put in one kind of starch-destroying enzyme, I know I will obtain maltose; if I put in another kind of enzyme, I will obtain dextrin, and so on. But, of course, a replicating system is by definition one that takes some copies of a specimen of the material that has to be copied and makes another copy of it, so the substrate has to determine what the final result is going to be if it is going to meet those conditions.

Twelve years ago, Kornberg had ample evidence that DNA-polymerase had these properties: that it was producing a product material whose properties were determined in great detail by the properties of the DNA that was used as the primer. This was about the time that he and I came to Stanford. I felt a great sense of excitement in having the opportunity to be able to look over his shoulder in the further development of this kind of biochemical investigation. I was confident that it was going to be a very short period of time before the use of Kornberg's polymerase on DNA, of the kind that I was accustomed to working with, which provided genetic information for the development and behavior of bacteria, would enable one to replicate bacterial genes in the test tube. From there one might go to the genetic ma-

terial of higher organisms, and all kinds of interesting experiments would be possible.

It has been rather surprising that it has taken 10 years from that date to actually accomplish the kind of result that was only recently published. The main methodological gap was the failure to recognize that there was another enzyme, whose existence was at the start not suspected, and which then had to be teased out and simultaneously studied by several different laboratories. This *repair* enzyme is capable of healing the nicks that appear in a growing molecule of DNA even if the DNA is by and large accurately replicated by the DNA-polymerase. These nicks are probably not accidental but are inherent in the detailed mechanism of DNA replication that result in small breaks in the growing DNA chain. If these breaks are not healed, the product DNA, which is obtained as a result of the action of DNA-polymerase on some primer DNA, will have most of the properties of the primer but will be a damaged copy. It will have breaks every few dozen or few hundred nucleotides down the chain. These are the elements of the DNA chain, and as a result, the copy DNA will not have biological activity when you test it for its genetic information; the sentences are broken.

Until this was recognized, it was quite a frustrating experience to attempt experiment after experiment, to obtain excellent chemical evidence that there had been a very neat replication of the primer information, to find that the new DNA that was synthesized depended on the primer DNA for its over-all composition, for its proportion of the bases, for the statistics of which element was placed next to which element, and that all of the low order chemistry of the product was exactly what you would expect in terms of the chemistry of the input material. But then when you test its biological activity, you find that it was essentially inactive.

About 2 years ago some of Kornberg's associates, and at Stanford I. R. Lehman's name must be especially mentioned, and a number of other laboratories elsewhere made almost simultaneous findings in this direction. They delineated the healing enzyme, polynucleotide-"ligase," the enzyme that can tie together broken strands, and can heal them together into one large continuous chain. In addition, a particularly appropriate experimental system was chosen: the genetic material of a very small virus that could be obtained in pure form and has a number of other technical properties that make these experiments much easier to do. The result was the demonstration that one could start with a specimen of the DNA of a certain virus phi-X-174, which happens to occur in nature in the form of a closed ring (and that represents one more reason why the healing enzyme was necessary, because in order to copy a ring, it has to be a string first and then finally closed up by the healing enzyme working at the head and the tail of it). To reuse that copy to make something exactly like the original would demonstrate that this had the biological activity of the original virus DNA. One of the technical properties that make that virus particularly suitable for this kind of experimentation is that under special conditions the virus DNA, by itself, is an infectious agent.

Ordinarily, the virus is released in the natural course of events with the DNA wrapped up inside a protein overcoat. This protects the DNA in its transit from the cell that it has infected and destroyed. It has been known for some time, however, that one could artificially strip off this protein overcoat and if one were extremely cautious in treating the free DNA, it could be allowed to unwrap itself. It is quite tightly wound up inside the virus coat, and under special conditions this isolated, purified, DNA is itself infectious and can start the process of virus infection. In that sense we can refer to the virus DNA by itself as being a living particle. It will initiate a life cycle of the virus when it is allowed to enter an appropriately sensitive bacterial cell.

A. T. Ganesan, in the Stanford genetics department has been pursuing similar lines of investigation, inspired by Kornberg's work. Ganesan reached substantially similar endpoints a few months behind Kornberg, with respect to the replication of other kinds of DNA. Thus, while the viral system is quite a specialized one, it really does represent an opening of the door into a very general application of the use of isolated enzymes for the replication of DNA in the test tube. We can take it for granted that there are only minor technical limitations with respect to the replication of biologically active DNA of any kind. That means that the copied material, the newly synthesized material, will have the same kind of biological activity that was represented in the specimen of DNA originally placed in the test tube.

"Creation" or Replication of Life

Many of the discussions center on this point, quibbling at the phrase "creation of life" (you know, is it really "living"?). Kornberg replied to that by saying that he had never given that question very much thought; there wasn't any great operational significance in whether one defined viral DNA as living or not, and I know this is correct. When he says he's not given much thought to it, I don't mean that he's thoughtless about it. Rather it is essentially a matter of taste at what level of complexity one wishes to draw the line in the definition of a living organism; my tastes happen to agree with his. I would have no hesitation whatever in describing a virus as a living organism, one that has very special nutritional requirements and only functions by being able to subvert the metabolism of another living cell that has a higher degree of autonomy.

However, I think there is a more appropriate point on which to quibble with those newspaper headlines. The title of Kornberg's paper in the proceedings of the National Academy of Sciences is not "Creation of Life in the Test Tube," but "Enzymatic Replication of the DNA of Bacteria Phage phi-X-174." The quibble is perhaps on the expression "creation" because the appropriate word is "replication." DNA has been put into the test tube, and the purpose of these investigations has been to discover the mechanism whereby DNA is replicated inside of living cells. Considerable insight had been achieved into this question by the investigations of 10 years ago. But at the time it was thought that the answer was in hand, whereas the crucial test was "Can

you make an effective replication of DNA information in a purified system, in the test tube, using the components that you think account for it inside the cell?" That didn't work. As long as it didn't, one was entitled to be quite skeptical about whether one had indeed accounted for the mechanism whereby DNA is replicated inside the cell. We now know that there was a very important missing ingredient: the DNA-polymerase. This enzyme that could be extracted and would accomplish the copying job certainly is the crux of the matter. Howevear, without the ligase and without certain other conditions, it is unable to function effectively in completing the cycle of replication of that information. I don't need to belabor the importance of this kind of insight in the most fundamental of biological mechanisms. There is now an enzymatic explanation for the way in which genetic information is copied from one generation to another. On the other hand, it is not quite appropriate to call it a creation. It is a replication. It is similar to the way in which the printing press works when it's making a number of copies of Shakespeare's sonnets. It is not the creation of those sonnets. The poetry in our analogy comes from the process of evolution during a period of a few billion years, to reach where all of us are today.

Khorana's Approach

What are the prospects for a creation in the more restricted sense that I have just discussed? The most active investigations along these lines are being pursued in the laboratories of Gobind Khorana at the University of Wisconsin in Madison.[1] He is an organic chemist, whereas Kornberg is an enzymologist. Khorana has been working on techniques for dealing with nucleic acids as organic molecules. He works with nonaqueous systems, with organic reagents, with the painful and plodding adding of one unit at a time to those long chains. When Khorana makes a polynucleotide, he is "creating" it because he starts out with a statement of the message that he wants to see represented in a DNA-like molecule. By dint of an enormous tour-de-force he ends up getting it. Now, we have a long way to go before the University of Wisconsin work can be regarded as a model of the creation of a gene. The longest chains that he has published on so far are about 30 units long, whereas the ones that the enzyme replicates many times a minutes, are several thousand units long. But he is getting there.

Now, in fact, the smallest nucleic acids that have a recognizable biological activity, that have a specificity that enables one to do a really interesting experiment with them involving a role in life, are about 80 units long. These are the nucleic acids, called transfer RNA, that are involved in transferring amino acid residues to the growing protein chain. They are not quite typical genes in the kind of specificity that they have, but they do represent a very attractive way station to motivate the work of the organic chemical laboratory. They help to prove that you can do it, that is to test out the precision with which you have copied the information that was in a certain sentence, in a certain book, in a certain encyclopedia, which is the blueprint of a large

organism. And by being one of the smallest sentences in that book, and by having a specific biological activity, they represent something more nearly feasible than some of the other tasks that you might set yourself to.

Khorana has divided the transfer RNA for phenylalanine into four parts, and he has set up four factories to attempt the assembly of the nucleotides needed for each of those four parts. He has worked out the methods with which those four parts can be grafted together; so I have no doubt that within the next few years he will have accomplished in vitro the organic chemical creation of a biological nucleic acid. This is perhaps a fifth of the size of the stretch of DNA that would be necessary to make a respectable gene, and these problems grow geometrically with the size of the product you are trying to make.

One might ask, "Is that creation?" Khorana has used not an existing DNA or RNA molecule and copied it with an enzyme, but he has gone through another step of abstraction. He has done a job similar to that which Holley was the first to report on, in working out the exact nucleotide sequence of a specimen of transfer RNA.[2] Then he wrote that down in a book, and now he is going to copy what he wrote down in a book rather than copy the original molecule. He is going to proceed by design rather than by direct impression. The contrast is a bit like getting one of Shakespeare's handwritten sonnets and using a photographic method to copy it in its original form, as opposed to having someone recite it over the radio as you write it down and then attempt to write or type it out again before putting it through the printing press. It will have been a symbolic transformation of the information that was in the original message, preceding the step of the organic chemist in assembling the characters of the message and putting the sentence together again.

That quibble, I think, is a reasonable one and should be thought about. The step of creation will arrive because once a chemist knows how to assemble *any* meaningful sentence, he can then really start creating. He can start putting together his own words, his own characters, and his own sentences. Most of them are going to be garbage in terms of some biological activity; even at the level of the transfer RNA there are some 4^{80} possible combinations. That number is rather larger than the size of the universe expressed in electron diameters, so it is not likely he'll get around to making all of them. But he will make a few, and I have no doubt that Khorana himself, one of these days, is going to create another transfer RNA molecule, a nucleic acid molecule that will have interesting properties, different from those of the naturally occurring material.[3] He will have studied nature closely; he will perhaps not like the sound of what he heard in the original poet's rendition of the sonnet and he will try to make up another one that does perhaps, some closely related job.

This clearly is a creation; it is based on some insight into the way that nature has worked, but we are certainly on the way there for messages on this size. From this one can guess that some brave soul is going to bludgeon the federal government into giving him $4 or $5 billion in order to proceed with

the task of synthesizing an even larger DNA sequence. Now, the job of manufacturing a poly-nucleotide sequence of the kind that we are describing, with the necessary precision at every step of the line (and you can't tolerate a 1 per cent error at each step or you'll have absolute garbage before you've gone 10 to 20 steps along), is a little bit like deciding that if I have a pick and shovel I know how to dig a hole, and therefore I'm going to start making the Grand Canyon. In principle that is absolutely right; but how many engineers are going to be motivated to want to participate in this particular kind of a program? I really have some doubts about it. I think it is necessary to parody this because I think there has been a lack of perspective about the actual significance of the results that have been achieved, which tends to obscure what really is going to happen.

Another analogy that I could give to the story would be to remark that I can still recall a headline announcement "Alchemists' Dream Achieved—Gold Synthesized in the Laboratory." That had something to do with the fact that a few atoms of the element gold had been fabricated in the cyclotron and someone had actually managed to produce enough of one of the radioactive isotopes of gold to be quite sure that that was his product so that he could justify the statement "creation of new forms of matter" as a legitimate headline. I don't think if that headline were to appear today it would allay the concerns of the United States government about our gold reserves. In principle, we could solve the problem of our external balance of payments by diverting our internal economic resources to the manufacture of gold by nucleonic methods, but I don't think that is likely to happen. It is extraordinarily important to distinguish the important leaps of insight that are represented by the things that can be done in principle because of the kind of understanding that they give. We have learned a great deal about gold by having manufactured it in the cyclotron and about the kind of practical utilities that are represented by saying, "This is the way that we're going to go about solving this or that technical problem."

HUMAN IMPLICATIONS

There are two specific limitations, both very, very important, in the human utility of biological advances such as DNA replication. The most important of these is that, at the present time, when it comes to an application in man, we don't really have any very useful application to make of the first sample of DNA, much less that of any copies that we might make of it in the test tube. DNA in the form in which it is isolated in the test tube has no known biological activity in man. I don't want to put too much emphasis on this, but it does represent a very important technical limitation to the application for good or bad of any of the other procedures that we are going to describe.

The way in which DNA functions in the human biological system is by being represented in a very highly organized form, being part of one of the chromosomes in one of the gametes, in the sperm or the egg. We have no insight, at the present time, as to how we would begin to approach the question of substituting one DNA molecule already present in a sperm or an egg with another DNA molecule that we had outside of it. And if we did, the questions of the mechanisms of replication, of creation of life in the test tube, and so on, would not have very direct *technological* bearing on it. They have a very important *intellectual* bearing on it, because the more we understand the difficulties that are involved in the other (and there's nothing like knowing what question you want to answer if you are trying to approach a solution to it), the better off we are. But at the present time, DNA in the form in which it is available in the test tube has no known biological activity. We don't know how to put it into a cell in such a way that it will influence the further development of that cell.

I am quite confident that this state of affairs will not last long, but the end of it will represent the threshold of the kinds of innovations that have been implied or foreseen, for example, by Senator Mondale, and might be the basis for his concerns. I will mention one way I think it might be accomplished. If we are talking about the specific design of DNA molecules, the one way we are not going to go about it is to attempt to put on a sequence of one nucleotide after another that is a thousand units long. In the long run this is not impossible. One could imagine a computer-driven machine that would do the job for you. It would certainly eliminate a good deal of the tedium that is involved in the manipulation of the reagents. Merrifield at Rockefeller University has been doing something quite similar to that with respect to protein synthesis, and a lot of the assembly-line effort that is involved in these kinds of manipulations could be avoided there.[4] It would still be an extraordinarily expensive procedure no matter how you wanted to do it, and there is no need to do it that way. Our concern, after all, is to produce, for experimental purposes, DNA molecules of varying composition, in order to see how they function when they are put into cells. Most of our investigations on this score are going to be done either in viruses or in bacteria because they are experimentally suitable material for looking at the way in which DNA works in the cell. We don't have to do a synthesis de novo of the DNA along Khorana's lines in order to accomplish this. All we have to do is to isolate the DNA that evolution has provided, take it from its natural sources, analyze it, and study how one sample of DNA behaves and then *introduce local, calculated, chemical changes* in the existing DNA.

This we have known how to do at one level or another for quite a long time. It is the process of mutation that has gradually been brought into more intelligible laboratory control. Even that, though, doesn't represent a very strong base of engineering performance, and there is just one other accomplishment I foresee occurring very soon that Kornberg's accomplishments will have led to. This will be grafting two DNA molecules together that have come from independent origins. For example, we might wish to study how a human gene functions in order to produce hemoglobin, one of the best-known human proteins. It would be nice to be able to take out the DNA that is appropriate to that function—in other words, extract DNA from human tissues, fractionate it, and look for the particular DNA molecules that have

that genetic information. This is essentially feasible today. Then we could graft that DNA molecule into the genetic information of the bacterium. (We do have methods where we can manipulate DNA and transfer it from one cell to another.) We should then end up with a bacterium that is making the pigment of the red blood cells of man, but that doing so under much more readily controllable conditions than some of the kinds of experiments that we wish to do. In fact, if we wanted to eventually engineer a better hemoglobin, we'd like to know what kinds of hemoglobins are going to be produced under different conditions. I would much rather begin with a hemoglobin-producing bacterium, produce mutants in it, look at the properties of the hemoglobin that it makes, and then eventually take out that DNA and put it back into where it might eventually have some human application, than have to do any of these intervening experiments with human material.

Now you could use tissue cultures, isolated cells of human or animal origin in a similar way, but we just don't quite know how to manipulate those cells at the present time for these kinds of purposes. How are we going to answer that first question, "Are there any prospects whatever of being able either to alter the existing genetic information that is in the sperm or egg or to add to it in the adult?" I have to place an unequivocal "no" with respect to the first point. I would view it as one of the last things we are likely to do in human experimental biology, to be able to point to a given gene in a sperm and introduce a change at that location from one quality to another. I'm not saying it will never happen. I'm saying that many other things, much more revolutionary in their impact on human affairs are likely to occur first. We might even have peace in the world before that time.

The technical complications of the kind of event that I have just described are really quite enormous, although one would be rather rash to say that it isn't going to happen tomorrow. There are always surprises in this game, but I think I can point to a considerable number of biological interventions that are just around the corner. Although I don't have space to dwell on all of them, I would like to mention one specific prospect.

To understand this story, we have to go back to bacterial genetics. The sorts of systems that we have studied experimentally in very simple organisms are cropping up again and again with potential applications in human affairs. This is the main message I want to get across. When you read about any biology in any organism, you must think to yourself, "Man is an organism that is part of life on the earth." There are just no rules whatever that will discount the application to cells of human beings of the kinds of principles that are applied to cells of other kinds of organisms. The essential quality of man is in his intellectual and social organization, and not the kind of body that he drags around with him, which is very much like that of the other animals. The route I have in mind stems from some of the most interesting experiments that I have ever done with a then-graduate student, now Professor Norton Zinder of Rockefeller University, about 15 years ago. They were not related to sexual recombination in bacteria, but to a totally surprising mechanism for exchanging genetic information between cells, namely, via virus particles.

Phi-X-174 probably doesn't do this, but a number of other viruses that attack bacteria have the quality that in addition to the genetic information that represents the virus itself (its ability to attack the cell), it has information with which to make more copies of itself, to make the protein overcoats, and to make the new enzymes that divert the cell's metabolism. It's easy for us to see why they are there in the virus nucleus; the genetic core of a virus should have the information to specify the pertubations from the normal life of the cell that the virus needs in order to take over. But besides that, there are any number of cases in bacteria where viruses also pick up, as passengers inside of that protein overcoat, other bits of genetic information in the cells that they are attacking. (We used to think that they just couldn't tell the difference, that any DNA that was lying around had a chance of being scraped up when the protein coats were being made; this may sometimes be true but the explanation is probably subtler than that some of the time.)

There was no obvious way in which to relate this to any human situation, although there was some speculation about it, until a few years ago when Stanfield Rogers at the Oak Ridge National Laboratory suggested that he was seeing, in a rather incidental way, some evidence of a similar process occurring in man. The Shope Papilloma virus was first studied by Shope at Rockefeller about 30 or 40 years ago. It is responsible for warts sometimes found on rabbits in the wild. It was one of the first examples where a virus could be demonstrated to be the cause of a tumor, in fact, in certain strains of rabbits it causes the tumors to grow much more aggressively than others, and it has been an interesting model for the way in which cancer might sometimes be produced by a virus. (The more we know about viruses, the more we know about cancer, and we are not likely to have a good answer to the cancer problem without the kind of information that studies on DNA have generated.)

The Shope virus has been studied in many laboratories for quite a long time, and no human has ever come down with a rabbit wart. There has been quite good evidence about the species specificity of this virus; it has been impossible to infect most other species with it and it has been regarded as quite an innocuous agent, unable to attack man. However, Rogers noticed that in tissue culture this virus infected cells of a wider variety of species than would be able to get warts from it. He didn't detect it by seeing any degenerative changes in the tissue culture; there was no direct evidence that the virus attacked these cells, but he did notice that there were certain enzymatic changes and that a particular enzyme called arginase, which attacks the amino acid arginine, was produced in cell cultures that had been infected with Shope virus.

During the last 5 years or so, there has been increasing evidence that tumor viruses can enter cells in culture or in the body and that these very specialized viruses that are sometimes capable of producing tumors in experimental animals seem to have the property of leaving behind some residue of their genetic information in the tumor cell line, although the virus as an infectious agent may disappear. The usual evidence that they have left something behind

There are other reasons why we should be surveying very large numbers of viruses for their biochemical properties. The reasons include the survival of the human species, because one of these days a virus is going to turn up that we don't have any defenses against. This will not merely decimate us (which means cutting off 10 per cent), it may wipe us out altogether. If you don't believe it, look what happened in Great Britain with hoof-and-mouth disease at the present time. We tend to be blind about such biological events until they happen. There is a great deal more about naturally occurring viruses that we should know on their own merits. Perhaps as a byproduct of this kind of search, we might develop a catalog of the viruses that inhabit the earth, of a kind that we don't have at the present time. This is not a very exciting enterprise for the biochemistry department or even the genetics department, but it is humanly important.

What can you do about it? I've already given you a hint, but rather than just look casually for a phenylalanine hydroxylase-producing virus just as a stab in the dark, for a virus that already happens to have this property, why don't we make one? It should be possible for us to isolate that fraction of the human DNA that has in it the coding for human phenylalanine hydroxylase. Nobody has quite done exactly that yet. But the results that have been obtained in bacterial systems are so close to it that it really is just a matter of effort to achieve success. Then we need only one more ingredient. We need to find one more enzyme (or other way) to graft phenylalanine hydroxylase DNA to the DNA of the Shope virus (since we know it works) and produce a synthetic virus that retains one important property of Shope, that it's innocuous. Most of its information is such that it did not lead to any observable alteration in human properties. Hence, we will now have this one new property, that is, the production of the new enzyme. From a technical standpoint, this is just around the corner. It does seem to me to be the most viable of the speculative opportunities in front of us for using DNA biochemistry for normative purposes, that is, for repairing obvious defects in man.

Now what stops us from making supermen? The main thing that stops us is that we don't know the biochemistry of the object that we are trying to produce. We have a long way to go before we arrive at that particular aim.

NOTES

1. Nobel Laureate awarded December 1968.
2. Nobel Laureate awarded December 1968.
3. Chemical modification of existing t-RNA molecules have recently been studied and have interesting properties.
4. J. Merrifield announced the laboratory synthesis of an enzyme, January 1969.
5. Still more recently, the chromosomes of such cells have been proven to carry new DNA, which resembles that of parts of the virus.

is that some of the immunological properties of the cells that they had infected have been changed. Sometimes the tumor transformation itself, the change from a normal-behaving to a malignant-behaving cell can be demonstrated to persist even in cell lines where you can no longer demonstrate the infectious virus, where you can no longer find particles that behave like the original virus particle. And yet this property is transmitted from cell to cell during the vegetative reproduction of these cells. More recently, there has been rather direct evidence that there is a deposit of this genetic residue because the RNA extracted from such cells shows certain new components in it, certain homologies with the viral DNA that indicates again that there has been something new added to the total DNA of the cells that have been infected.[5]

The new point that Rogers brought out was that when he examined the blood of a number of humans, research workers who had been studying the Shope virus, he found a rather consistent pattern of greatly reduced levels of blood arginine. The statistics on these are fair and the conclusion seems almost inescapable that a harmless infection with the Shope virus, that is, in human cells the Shope virus does not have any tumor-inducing property, but that it has entered some of the cells of these people and has provoked there some of the new enzymes that the virus is capable of inducing in cells in tissue culture. He commented that this was a modification of the development of these humans in a rather trivial way, because there is no observable clinical difference between these people and anybody else. They are not suffering from any obvious disease; they get by very well with these low arginine levels, but he pointed out that somebody in the world may be suffering from a disease that needs arginase to cure it! Arginase is a naturally occurring enzyme in the liver that plays a very important role in ammonia metabolism. So far, no humans have been found living who lack that liver arginase, probably because they couldn't survive very long. Here is a case where there is a therapy in search of a disease, because Rogers proposes to remedy the genetic effect of the hypothetical arginase-deficient human, who might exist on the basis of having some damage to his own genetic apparatus in the part that codes for arginase, by giving him Shope virus, and it really ought to work on these principles. But he doesn't stop there (here, of course, is the exciting extrapolation). If we looked at a large number of other viruses, we will surely find viruses that induce other enzymes. And we ought to be on the look-out for one that takes care of the enzymes for phenylalanine metabolism. Now here is a disease that already exists; and while there is a therapy for it, it is a rather clumsy one. The disease is phenylketonuria, PKU, which is a rather distressing thing to happen to a child. It is a genetic disease, the inability to make the enzyme needed for the metabolism of phenylalanine; the phenylalanine that he gets in his diet piles up in his blood, and in some mysterious fashion inhibits the proper development of his brain. By putting these kids on very unappetizing diets, which are limited in phenylalanine, it is now possible to control the disease, but it would really be very much nicer to have some built-in phenylalanine oxidase, and if we just had the right virus we could deal with it.